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PRELIMINARY INVESTIGATION OF THE ROLE OF AQUATIC MACROPHYTES IN HEAVY METAL UPTAKE FROM WATER WITH COAL PARTICLES

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FOREWORD

Attempts to predict the environmental impacts of greatly increased utilization of western coal clarified the need for data on the effects of each segment of the total coal-based fuel cycle, from the mine through transport, storage and conversion to electrical energy. In order to partially meet the need, a study was made of the environmental effects of the storage of western coal in large open-air holding piles in a transshipment facility located in the Superior, Wisconsin - Duluth, Minnesota harbor area.

This report presents the results of studies of the amounts of metals leached from a western and an eastern coal and the effects of these metals on several aquatic plant and invertebrate organisms.

Norbert A. Jaworski, Ph.D.
Director
Environmental Research Laboratory-Duluth

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ABSTRACT

A survey was made and representative samples of vascular macrophytes from the Superior, Wisconsin-Duluth, Minnesota Harbor were collected in August 1975 and stored in a frozen condition until processed. The processed samples were analyzed for environmentally sensitive metal concentrations by flameless atomic absorption spectrophotometry.

The potential of using Lemna minor L. to examine the effects of Western coal upon growth was investigated. Lemna minor L. was grown in Lake Superior water in the presence of incremental amounts of particulate coal. Temperature, humidity and light were controlled and growth rates were determined by plant frond counts. Samples of Lemna were collected for metal uptake analyses. Preliminary evidence suggested that coal particulates, at certain concentrations, may diminish the growth of this aquatic plant. Increased metal uptake by the plant was noted.

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SECTION 1

INTRODUCTION

Heavy metals are frequently sequestered by plants, and if these plants are important in the food chains of an area, they may serve as a source for the introduction of the same heavy metals into higher level consumers. In addition, heavy metals may be moved from the environment to the detritus food chain only to be flushed back into the water. In examining the effect of the coal transshipment facility on the addition and dispersion of heavy metals throughout the Superior-Duluth Harbor, it was desirable to obtain some preliminary estimates of background levels of the metals under study in the aquatic vascular macrophytes which were present in the harbor.

Although many heavy metals ions are necessary for normal plant growth, excessive levels of heavy metals may be toxic. The addition of coal particles to the water of the Duluth-Superior harbor raises the possibility that plants may be affected. In order to examine that possibility, initial studies to evaluate the feasibility of establishing plant cultures for studying the uptake of heavy metals from coal particulate additives was undertaken. In addition, a first approximation of the relationship between growth and coal additives was sought.

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SECTION 2

CONCLUSIONS

Results demonstrated that the different species concentrated the different metals not only in differing amounts, but also that the various parts of the plant had differing levels of metals present. Some plants (such as Fissidens grandifrons Brid) appear to have high levels of selected metals.

Preliminary work suggests that Lemna minor is a useful plant to study uptake of heavy metals. It does not require elaborate culture techniques and its small size allows smaller (and less expensive) culture facilities.

Although the work reported above reflects only a first approximation in examining the role of plants in heavy metal uptake from coal particles, there is evidence that coal particulates may, in certain concentrations, diminish the growth of an aquatic plant. In addition, there appears to be increased uptake in the presence of coal particles. Certain metals (such as barium) seem to be increasingly sequestered from the media with increasingly higher amounts of added coal. Others (Zn, Pb) show greater levels of accumulation at intermediate concentrations of coal additive. However, other metals (cobalt, copper, manganese) exhibit no marked trends.

SECTION 3

RECOMMENDATIONS

A continual monitoring program of selected plant species in the harbor would be a useful tool in judging the role of plants in heavy metal uptake within the Superior-Duluth Harbor ecosystem. The high levels present in species such as the aquatic moss Fissidens grandifrons needs further study, particularly to assess the potential of contamination from extraneous dust.

An aquatic system using Lemna minor as a test species provides a good means to assess metal uptake but considerably more work remains to be done in understanding the nature and extend of this uptake before assessing its significance. The somewhat erratic results which are reported suggest the potential of contamination in the system as described.

SECTION 4

BACKGROUND LEVELS OF HEAVY METALS IN HARBOR AQUATIC MACROPHYTES

SAMPLE COLLECTIONS AND PREPARATION

Samples of the aquatic macrophytes were collected in August 1975 from areas in the Superior-Duluth Harbor near the coal transshipment facility construction site. The samples were rinsed with distilled water to remove surface contaminants and then placed in acid-washed polypropylene bottles. The specimens were then frozen and stored in a frozen condition until processed for metal analyses.

Representative portions of the stored specimens were oven dried to constant weight, digested in an ignition bomb by means of ultrapure nitric acid, and then metal concentrations were determined by flameless atomic absorption spectrophotometry.

Samples, in the size range of 0.08-0.10 g, were accurately weighed on an analytical balance. The weighed samples were then placed in Parr® Teflon® lined acid digestion bombs and 2.50 to 3.00 ml of ultrapure, concentrated nitric acid were added. The acid digestion bombs were then assembled, sealed and heated in an oven at 150°C for a period of two to three hours.

The digestion bombs were removed from the oven and allowed to cool to room temperature. The bombs were then opened and the contents were quantitatively transferred to volumetric flasks and diluted to a standard volume with deionized water.

Samples, after digestion, were analyzed for metal content by the use of flameless atomic absorption spectrophotometry.

Full details of preparation and analysis of the coal particles are presented by Coward and Horton, (1980).

ANALYSIS

Results of the preliminary population survey are shown in Tables 1 and 2. Table 1 is a record of the species collected, and the portion of the plant which comprises the analytical sample. Table 2 gives the results of the various analyses for the metals under consideration.

Results demonstrated that the different species concentrated the different metals not only in differing amounts, but also that the various parts of the plant had differing levels of metals present. Perhaps most unusual were the levels of lead and manganese sequestered by the aquatic moss, Fissidens grandifrons Brid. These high levels are worthy of further study, particularly to determine if the concentration of these metals is a result of the substrate from which they were collected, or if the species under consideration has an unusual facility for sequestering these metals (lead and manganese). Also noteworthy are the relatively low levels of metals in wild rice (Zizania aquatica L.), which is one of the few aquatic macrophytes in the area now directly eaten by man.

Many of the other species investigated may be eaten by ducks or fish, and in turn consumed by man (Fassett, 1960). Thus, the levels of environmentally sensitive metals in these aquatic vascular macrophytes may eventually be a matter of concern to the region.

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TABLE 1. SPECIES AND HABITATS MONITORED FOR BACKGROUND LEVELS OF HEAVY METALS IN THE DULUTH-SUPERIOR HARBOR AREA.

Sample Number	Taxon	Portion Analyzed	Habit
1	Lemnar minor L.	Whole Plant	Free-Floating
2	Fissidens grandifrons Brid. (a moss)		Attached to Wood Piling
4A	Bidens cernua	Stem	Emergent
4B		Leaves	
		Flower	
5	Ceratophyllum demersum L.		Submerged
7	Scirpus validus Vahl.	Rhizome	Emergent
8		Root	
9		Leaf	
10	Nuphar variegatum Engelm	Whole Plant	Floating Leaved
11	Zizania aquatica L.	Sead	Emergent
12		Root	
13A		Stem	
13B		Leaf	
14	Bidens beckii Torr.	Leaf	Submerged
15		Root	
16	Sparganium chlorocarpum Rydb.	Root	Emergent
17		Flower	
18		Leaf	
19	Sagittaria latifolia Willd.	Root	Emergent
20		Leaf	
21A		Stem	
21B		Flower	

TABLE 2. CONCENTRATION OF METALS (ppm) IN CERTAIN DULUTH-SUPERIOR HARBOR PLANTS (BY DRY WEIGHT)

Sample	As	Ba	Cd	Cr	Co	Cu	Pb	Mn	Mo	Ni	Se	V	Zn
1	1	237	0.4	4.9	6.5	15	29.4	5000	1.5	23	0.8	5	16
2	11	528	0.8	14.8	15.0	32	198.8	15400	<1.5	11	<0.1	8	9
4A	<1	25	0.2	0.9	0.7	12	4.7	653	--	39	--	--	10
4B	<1	95	0.1	1.8	4.6	17	2.2	2080	--	3	--	--	8
4C	<1	23	0.1	0.9	1.0	13	2.0	1170	--	3	--	--	5
5	5	604	0.8	4.1	5.7	106	30.5	746	--	11	--	--	11
7	--	4	--	<0.1	--	2	0.4	102	--	2	--	--	3
8	12	20	0.5	1.9	6.9	7	11.5	2040	--	6	<0.1	--	10
9	<1	78	0.1	0.5	1.9	2	3.3	962	1.2	4	0.3	<4	3
10	<1	367	0.3	1.7	5.0	28	78.8	618	<2.1	79	1.2	<5	23
11	<1	<7	0.1	0.4	8.8	11	2.4	250	--	4	1.6	<5	8
12	5	50	0.1	2.5	5.4	9	8.8	573	--	14	--	--	9
13A	8	176	0.5	11.7	9.0	208	23.5	781	--	59	--	--	22
13B	<1	87	0.3	3.2	5.5	31	13.3	353	--	12	--	--	18
14	<1	112	0.2	1.3	2.3	8	3.8	1250	--	2	--	--	4
15	6	95	0.2	2.5	3.5	24	10.4	1570	--	2	--	--	6
16	6	64	0.1	1.8	5.8	10	11.8	1520	--	35	--	--	7
17	<1	<3	0.1	0.3	2.4	3	1.4	90	--	1	--	--	7
18	<1	50	0.1	0.8	2.8	18	23.1	254	--	29	<0.2	--	8
19	10	479	0.4	5.5	1.0	18	15.9	479	--	5	--	--	21
20	<1	18	0.1	0.4	1.6	8	1.8	261	--	6	--	--	5
21A	<1	<9	0.2	0.6	1.4	36	3.1	263	--	6	--	--	11
21B	1	<4	0.1	0.6	0.6	85	4.5	168	<1.2	1	0.2	<3	6

SECTION 5

EFFECTS OF PARTICULATE COAL ON *LEMNA MINOR* GROWTH

BACKGROUND

The effects of heavy metals on plants has been studied in the context of agriculture (such as Millikan, 1949) and, to a lesser extent, in the context of polluted terrestrial substrates (such as Alloway and Davies, 1971). Studies also have been done with marsh plants, particularly *Spartina alterniflora* (Dunstan and Windom, 1974) but little work exists with aquatic plants.

Many metal ions have been found to be essential for normal plant growth and function. Manganese was found by early workers (Epstein, 1972) to be required. The role of many such elements has been shown in enzyme activation, regulation or stimulation. Epstein has reported (1972) that, in addition to its primary function, manganese served as an activator of some enzymes by substituting for magnesium in certain phosphate transferring enzymes. The same author also reported that manganese, if present in high concentration, induced iron deficiency in some plants. Treshow (1970) demonstrated that certain trace elements and/or alien metal ions could induce metal deficiencies or be directly toxic to plants.

In view of the relatively low levels of nutrients and dissolved ions in the waters of Lake Superior, addition of even small amounts of coal, from which a variety of materials can leach, is potentially disruptive. The addition of coal particles to the aquatic environment would presumably have an effect on the aquatic vascular macrophytes, depending on the amount of coal present. The presence of coal could result in increased growth (through the addition of nutrients) or increased environmentally sensitive metal uptake from the coal-enriched surroundings (which could result in growth inhibition at excessive metal levels).

The second phase of this aspect of the study was to investigate the feasibility of studying heavy metal uptake in vascular aquatic plants. First year efforts were directed toward selecting a study species and determining a potentially useful procedure. Within the limitations of the budget and time allocation of the first year (and in anticipation of further work in subsequent years), it was possible to make only preliminary attempts to answer the major questions. Initial efforts were focused upon a study of the effect of coal upon *Lemna minor* L.

Lemna minor L. was selected as the plant for these initial studies because of its small size, relatively rapid growth and structural simplicity. Lemna minor L. produces new daughter fronds from pockets at the base of the parent plant and these new plants may, in turn, produce daughter fronds even before they are detached from the original parent. Growth may be followed by counting these fronds, a procedure which has proven to be quite reliable (Hillman, 1961).

Another factor which lead to the adoption of Lemna minor L. as the test organism was the extensive experimental literature on Lemna sp. (see Hillman, 1961, for a review of the description and use of the Lemnacae in experimental procedures).

PROCEDURES

Field collection of Lemna minor L. were made from indigenous populations, rinsed with deionized water to remove any gross contamination, and reared in acid-washed aquaria using Lake Superior water as a culture medium. From the above culture, groups of twenty-five (25) unattached Lemna minor L. fronds (without daughter fronds) were removed, rinsed three times with deionized water and placed in 600 ml acid-washed polypropylene beakers which contained 300 ml of Lake Superior water.

Western Coal No. 1 was used in studying effects on Lemna minor L. A report of the properties of the Decker coal delivered to the Superior-Duluth area may be found in the Environmental Impact Statement by Roy F. Weston, Inc. Train-load composite figures on representative trace metal concentrations (in ppm) for this coal are as follows:

As	2.30	Co	3.70	No	4.80
Ba	170	Cu	15.0	Ni	3.00
Cd	25.0	Pb	0.39	Se	1.00
Cr	10.0	Mn	41.0	Zn	0.58

A portion of this coal was ground in the Wiley® mill and then added to the beakers of Lemna minor L. culture. The amount of coal added to each beaker (of 300 ml water) was 5.00 g, 10.00 g or 25.00 g. Triplicate sets of Lemna minor L. culture samples were prepared with each size of coal adduct.

The beakers were covered with acid-washed glass plates and placed in an environmental chamber in which temperature was held constant at 25°C. A sixteen-hour light period at an intensity of 1200 foot-candles (12,900 lumens/square meter) alternated with eight hours of darkness. Growth was allowed to proceed for 21 days. Water lost through evaporation was replaced with deionized water.

The lake water culture medium was sampled prior to the start of the growth chamber study, and all growth media which had been in contact with coal were sampled at the end of the 21 day growth period. The samples were acidified with ultrapure nitric acid and were stored in acid-washed polypropylene bottles until analysis for metal content were performed. In addition,

samples of the Lemna minor were taken from the population for metal analyses at the initiation of the run, as well as from each culture at the conclusion of the test period.

At the end of the growth, the Lemna were harvested (after counting), dried to constant weight, and the residue analyzed for its metal content.

Individual counts of all plant fronds (as previously described) were made at intervals, to determine growth rates and to observe the plant growth response to the presence of coal derived materials in the Lake Superior water growth medium.

RESULTS

Initial efforts in the laboratory phase of this study were directed toward establishing procedures for the culturing of Lemna minor L. in media which was fortified by the addition of ground coal in various concentrations. As a result, time was not available for the adequate retesting of the results tabulated in this report. Inspection of these data showed no consistent patterns. Because of the preliminary nature of the data (and the inherent variance), it was felt that no statistical tests were warranted.

A summary of the growth response of Lemna minor to varying concentrations of ground coal present in the lake water medium is presented in Table 3.

TABLE 3. Growth of Lemna minor L. in Coal Fortified Lake Water (Average Frond Counts of Three Trials)

Medium (300 ml)	Number of Fronds by Day								
	1	3	7	10	13	16	20	22	23
Lake Water, No Coal Added	25	52	128	184	215	243	140	147	---
Lake Water, 5.00 g Coal Added (1.67% coal, by weight)	25	61	146	182	191	218	169	193	---
Lake Water, 10.00 g Coal Added (3.33% coal, by weight)	25	71	126	163	195	241	232	---	267
Lake Water, 25.00 g Coal Added (8.33% coal, by weight)	25	61	148	188	200	235	228	---	256

Table 4 presents the growth rate constants as determined from frond counts (Hillman, 1961). The first three days are omitted on the assumption that they represent more nearly the conditions existing in the original culture than that of the experimental media. Frond count data after 16 days are also omitted from this table since frond counts for unfortified lake water

and water with 5.00 grams of added coal are declining--perhaps due to lack of nutrients.

TABLE 4. Growth Rate Constants for Lemna minor L. in Coal Fortified Lake Water (Average of Three Replicates)

Medium (300 ml)	Interval (Days)			
	3-7	7-10	10-13	13-16
Lake Water, No Coal Added	.098	.053	.023	.018
Lake Water, 5.00 g Coal Added (1.67% Coal, by weight)	.095	.033	.007	.019
Lake Water, 10.00 g Coal Added (3.33% Coal, by weight)	.083	.037	.026	.031
Lake Water, 25.00 g Coal Added (8.33% Coal, by weight)	.096	.035	.009	.023

Table 5 presents data for the average metal concentration in the lake water culture media (with and without coal) at the beginning and end of the 21 day growth test with Lemna minor. Table 6 contains data for the average metal concentration in the Lemna grown in the above culture media.

The use of unfortified lake water alone as the medium poses some difficulties since the level of available nutrients is low. However, within the harbor ecosystem, uptake of these metals by aquatics is likely to be limited by similarly low nutrient levels. Further work to relate uptake with the nutrient level of the growth media would be useful.

This work involved only static testing, simulating coal accumulation at the bottom of the water column. Since water currents are present, much coal, especially fine particles, could be held in suspension for periods of time, increasing the likelihood of leaching. In addition there is need to better understand the effect of particle size upon leaching rate which should be reflected in uptake rates.

The significance of metal concentrations reported in Table 6 must be interpreted with extreme caution. It is hard to explain the variance present without a much greater understanding of the behavior of each metal tested. The possibility of error in metal determinations, particularly due to the small amount of tissue available, must be considered.

In addition, the bioaccumulation of all metals tested (such as Ba, Co, Mn, V and Zn) are not expected and may suggest contamination, either in the culture or in the later chemical analysis.

TABLE 5. HEAVY METAL CONCENTRATION, ppb, IN THE WATER CULTURE MEDIUM BEFORE AND AFTER A 21 DAY *Lemna minor* GROWTH PERIOD WITH VARYING CONCENTRATIONS OF COAL ADDITIVES (AVERAGE OF THREE REPLICATES).

	As	Ba	Cd	Cr	Co	Cu	Pb	Mn	Mo	Ni	Se	V	Zn
Initial Lake Water Levels	0.0	3.3	0.00	0.0	0.0	1.30	0.70	0.4	0.00	0.7	0.20	0.0	0.4
Water Levels After 21 Days of Growth													
No Coal Additive	0.0	0.0	0.08	0.1	0.0	1.80	0.00	0.1	0.00	20.5	0.30	0.0	1.4
5.00 g Coal Added	0.0	1.1	0.05	0.1	0.2	0.10	0.30	0.1	0.60	3.7	0.00	1.0	1.9
10.00 g Coal Added	0.1	2.2	0.05	0.0	0.2	1.60	0.10	1.1	2.32	0.0	0.00	1.9	0.8
25.00 g Coal Added	0.0	3.3	0.07	0.1	0.2	1.10	0.06	1.0	2.10	7.1	0.95	0.0	0.1

TABLE 6. HEAVY METAL CONCENTRATION, ppm (BY DRY WEIGHT BASIS) IN Lemna minor L. GROWN IN DIFFERING LEVELS OF COAL-FORTIFIED MEDIA (AVERAGE OF THREE REPLICATES).

	As	Ba	Cd	Cr	Co	Cu	Pb	Mn	Mo	Ni	Se	V	Zn
Initial Concentrations in Lemna	0.30	10.0	1.9	0.5	0.30	40.0	0.8	33.0	1.6	8.0	8.0	4.0	50.0
Final Concentrations in Lemna													
Lake Water, No Coal Added	0.70	11.0	0.4	0.4	0.70	14.0	41.2	257.0	3.4	9.0	0.5	8.0	17.0
Lake Water, 5.00 g Coal Added	0.80	161.0	0.7	0.7	2.20	20.2	4.1	117.0	4.3	10.0	0.6	11.0	28.0
Lake Water, 10.00 g Coal Added	0.80	210.0	0.8	2.9	1.05	81.0	7.7	268.0	4.2	19.0	0.7	11.0	42.0
Lake Water, 25.00 g Coal Added	1.33	114.0	1.6	0.8	1.30	21.0	16.0	143.0	6.7	46.0	13.9	17.0	62.0

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